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## Introduction

- Wild mustard (*Sinapis arvensis* L.) is a severe annual weedy species in croplands in Canadian Prairies that produces persistent dormant soil seed bank. Seeds contain high percentage of toxic alkaloids lethal for livestock if grazed in excess.
- Smoke produced during the burning of plant materials has shown promotive action on Brassicaceae family seeds' dormancy break & germination.
- Smoke water application to soil, possibly can break the dormancy and induction of synchronous germination of soil-born Wild mustard (WM) seeds facilitating for efficient secondary weed control methods. i.e. Herbicides
- A recent study showed that alfalfa-derived smoke trigger seed germination in native fescue prairie species.
- No study has been done on Wild mustard seeds' germination response with alfalfa smoke solution.



Fig. 1 Wild mustard (*Sinapis arvensis* L.) seeds

## Hypothesis

Alfalfa smoke solution have special chemical compound that can break seed dormancy of Wild mustard seeds during dark germination.

## Objectives

To isolate active compound/s in Alfalfa smoke solution that can break seed dormancy of WM seeds during dark germination.

## Materials & Methods

- Treatment solution = 'smoke-water solution' from plant burning



Fig. 2 Smoke-water producing setup (Kernan Farm) where smoke from smoldering plant passing via distilled water containers

Table 1 Treatments and seeds used in growth incubators

Variables	No. of Levels	Levels
Smoke Type	08	SA <sub>1</sub> -SA <sub>6</sub> , alfalfa, wheat
Smoke dilutions	05	DW, 1/1000, 1/100, 1/10, 1/1 (v/v)
Light condition	02	Light (12h/12h) & fully dark(24h)
Temperature	02	25°C or 25/15°C
Seed species	02	lettuce ( <i>Lactuca sativa</i> L. cv. Salad Bowl) & wild mustard (WM)

DW: distilled water (control), SA: South African 06 Species (Fig. 1)

- 1) Salad Bowl lettuce bioassay (Standard germination test for smoke solutions)
  - 4 mL smoke solution/petri plate, all variable in Table 1
  - Fig. 2 (a) and (d)
- 2) WM seed priming and Germination test
  - Soak-primed in 10 mL of alfalfa smoke dilutions in dark for 24hr, 20°C
  - 01 week drying at 25°C, 4 mL DW, 20°C (16h L/8h D) or full darkness for 10 days
- 3) Tetrazolium seed viability test (ISTA,2003)
- 4) Actives separation with non-polar FPX-66 resin & NMR analysis
  - 49 g of resin for 1 L of smoke solution, wash with 10% & 100% ethanol

All laid in CRD. Final germination (G%) analysed in GLM (Poisson distribution and log link function in R). Treatment means were separated using Tukey test at P<0.05.

## Results

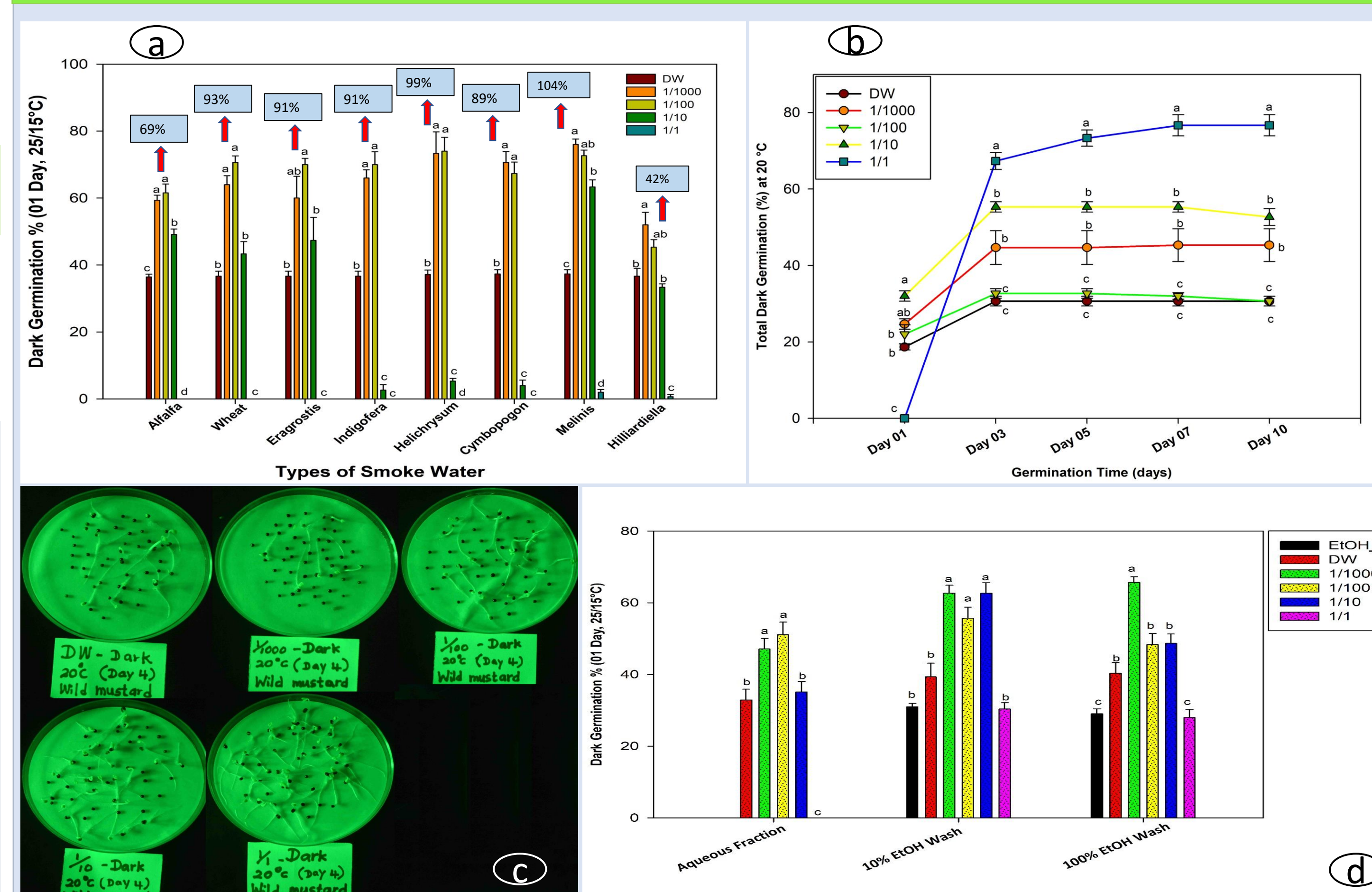


Fig. 3 (a) G% of lettuce seeds vs smoke types (25/15 °C-24h dark -1 day). (b) & (c) G% of WM seeds after smoke priming (20 °C-24h dark -10 day). (d) G% of Lettuce seeds after treat with Resin fractions. Means with different letters indicate significant difference (P<0.05) within each smoke types. Error Bar = mean ± SE

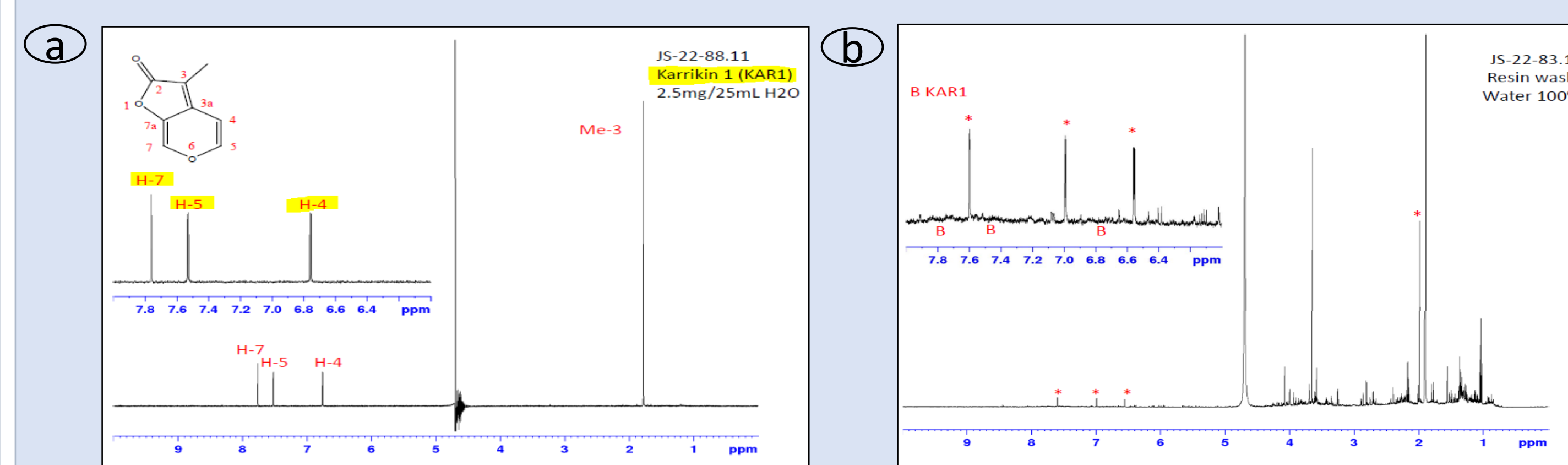


Fig. 4 Comparative 1D <sup>1</sup>H NMR spectra obtained from (a) standard Karrikin and (b) alfalfa aqueous smoke water after resin adsorption

- Smoke action on both seeds is significant (P<0.05) in fully darkness (data not shown) similar to the real soil seed bank condition. Fig. 3 (a) – Irrespective of smoke types, smoke increase the dark seed germination significantly (P < 0.05) compared to DW control.

## Results

- The 25/15 °C alternative temperature significantly (P<0.001) increases the smoke responses of lettuce seeds by all smoke types compared to constant 25 °C (data not shown).
- Fig. 3 (b) – Except 1/100 v/v, all other smoke dilutions increase the G% of WM seeds (P<0.05). (d) - all three fractions significantly increase lettuce seed dark germination (P<0.05) compared to DW.
- Fig. 4 (b) – active compound Karrikin (KAR1) is present in 100% aqueous fraction as confirmed by 1D <sup>1</sup>H NMR spectra.

## Discussion & Conclusion

- In fully darkness & 25/15 °C alternative temperature, lettuce seeds significantly (P<0.001) response to alfalfa smoke indicating that smoke water chemicals can replace the light requirement for germination.
- After 03 days, 1/1 v/v dilution-primed WM seeds showed the highest significant (P<0.001) germination which is increased by 120%.
- This type of "suicidal germination" of WM seeds primed with alfalfa smoke, can be exploited to eradicate the dormant weed soil seed bank in crop lands. Stimulated weed seedlings can be controlled either by mechanical and/or herbicide applications.
- Nonpolar FPX-66 resin absorbs nonpolar compounds such as organic polysaccharides, acidic compounds etc. KAR1 is the most potent seed germinating 'plant growth regulator' isolated from smoke water (Flematti *et al.*, 2004). FPX-66 resin is possibly effective in isolating KAR1.
- This study revealed that in fully darkness, dormant Wild mustard seeds primed with alfalfa smoke solution (or KAR1) can break dormancy and cause "suicidal germination" even at higher smoke concentration (1/1 v/v). Further research is essential to explore it's true potential on weed soil seed bank in field settings.

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